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INTRACELLULAR pH AND ITS RELATIONSHIP TO REGULATION OF ION TRANSPORT IN NORMAL AND CYSTIC FIBROSIS HUMAN NASAL EPITHELIA

BY NIELS J. WILLUMSEN AND RICHARD C. BOUCHER

From the Zoophysiological Laboratory A, August Krogh Institute, University of Copenhagen, Universitetsparken 13, DK-2100 Copenhagen Ø, Denmark, and the Division of Pulmonary Diseases, Department of Medicine, School of Medicine, The University of North Carolina, Chapel Hill, NC 27514, USA.

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SUMMARY

1. Intracellular pH (pH_i) of cultured human airway epithelial cells from normal and cystic fibrosis (CF) subjects were measured with double-barrelled pH-sensitive liquid exchanger microelectrodes. The cells, which were grown to confluence on a permeable collagen matrix support, were mounted in a modified miniature Ussing chamber. All studies were conducted under open circuit conditions. Values are given as means ± S.E.M. and n refers to the number of preparations.

2. Normal preparations ($n = 15$) were characterized by a transepithelial potential difference (V_t) of -18 ± 2 mV, an apical membrane potential (V_a) of -19 ± 2 mV, a basolateral membrane potential (V_b) of -37 ± 2 mV, a transepithelial resistance (R_t) of $253 \pm 15\ \Omega\ cm^2$, a fractional apical membrane resistance (fR_a) of $0{\cdot}40 \pm 0{\cdot}04$ and an equivalent short circuit current (I_{eq}) of $-73 \pm 7\ \mu A\ cm^{-2}$.

3. CF preparations ($n = 13$) were characterized by a V_t of -46 ± 7 mV, a V_a of 3 ± 5 mV, a V_b of -43 ± 3 mV, R_t of $373 \pm 47\ \Omega\ cm^2$, fR_a of $0{\cdot}44 \pm 0{\cdot}04$ and an I_{eq} of $-130 \pm 16\ \mu A\ cm^{-2}$. All parameters except V_b and fR_a were significantly different ($P < 0{\cdot}025$) from those of normal preparations.

4. Despite large differences in electrochemical driving force for proton flow across the apical cell membranes between normal and CF preparations (-4 ± 3 mV and 20 ± 7 mV, respectively), pH_i was similar ($7{\cdot}15 \pm 0{\cdot}02$ and $7{\cdot}11 \pm 0{\cdot}05$, respectively). The driving force across the basolateral membrane was similar in normal and CF preparations (22 ± 3 and 26 ± 3 mV, respectively).

5. Intracellular alkalinization achieved by removal of CO_2 from the luminal Ringer solution or by luminal ammonium prepulse led to stimulation of I_{eq} in both normal (from -58 to $-70\ \mu A\ cm^{-2}$, $n = 4$; $P < 0{\cdot}05$) and CF (from -144 to $-163\ \mu A\ cm^{-2}$, $n = 4$; $P < 0{\cdot}005$) preparations. The increase in I_{eq} was associated with a reduction of R_t, increase in fR_a, and hyperpolarization of V_b. All changes in bioelectric properties in response to intracellular alkalinization were fully reversible.

6. Intracellular acidification achieved by serosal ammonium prepulse led to marked reductions of I_{eq} in both normal (from -95 to $-31\ \mu A\ cm^{-2}$, $n = 6$; $P < 0{\cdot}05$) and CF (from -111 to $-67\ \mu A\ cm^{-2}$, $n = 7$; $P < 0{\cdot}005$) preparations.

The decrease in I_{eq} was associated with increase in R_t, decrease in fR_a, and depolarization of V_b. All changes in bioelectric properties in response to intracellular acidification were fully reversible.

7. Recovery of pH_i following intracellular acidification occurred faster in CF (14 ± 3 min, $n = 8$) than in normal preparations (45 ± 6 min, $n = 6$).

8. The pH_i was not affected by exposure to luminal Na^+-free Ringer solutions (*N*-methyl-D-glucamine substitution). Luminal exposure to high amiloride concentrations (10^{-3} M) did not affect pH_i under control conditions. Amiloride (10^{-3} M) did not affect pH_i recovery either when the cells had been pre-acidified by exposure to 20 mM-NH_4Cl (ammonium prepulse) in the serosal Ringer solution for 4 min.

9. We conclude that pH_i is maintained at a normal value in CF airway epithelial cells and that in both normal and CF preparations, pH_i regulates basolateral membrane potassium conductance. The observed variation in pH_i and the associated modulation of potassium transport are likely to take place *in vivo* during a full breathing cycle.

INTRODUCTION

Intracellular pH (pH_i) is an important intrinsic factor controlling a multitude of cellular functions (Roos & Boron, 1981), and it has the potential to act as a rapid signal for co-ordination of intracellular processes. Cellular mechanisms that contribute to regulation of the free intracellular activity of protons include metabolic production of protons and bicarbonate, intracellular buffer systems, and specialized transport mechanisms for these ions, e.g. Na^+–H^+ and Cl^-–HCO_3^- exchangers, proton ATPases, and conductive pathways for H^+ and HCO_3^- (Harvey & Ehrenfeld, 1988*a*, *b*).

Intracellular pH has been reported to affect the function of major cation transport systems in mammalian epithelia, e.g. basolateral K^+ channels in bovine retinal pigment epithelium (Keller, Jentsch, Koch & Wiederholt, 1986), outer renal medulla (Klærke, Karlish & Jørgensen, 1987), and rabbit distal colon (Duffey & Devor, 1990). In frog skin epithelium apical Na^+ channels and basolateral K^+ channels are both regulated by pH_i (Harvey, Thomas & Ehrenfeld, 1988). Also the Na^+–K^+ pump in rabbit urinary bladder is reported to be pH_i dependent (Eaton, Hamilton & Jones, 1984). From these data, it has been concluded that pH_i acts as a signal that co-ordinates the activities of the apical and basolateral ion transport systems (Harvey *et al.* 1988), a phenomenon originally described as 'cross-talk' by Schultz (1981), Diamond (1982), and Davis & Finn (1982).

Airway epithelia may be exposed to unique conditions that affect pH_i and its regulation. First, large variations in luminal CO_2 tension (P_{CO_2}) occur on the luminal surface of airway epithelia during inspiration ($P_{CO_2} \approx 0$ Torr) and expiration ($P_{CO_2} \approx 40$ Torr). The effects of phasic changes in P_{CO_2} on pH_i and the homeostatic mechanisms in airway cells that may respond to these changes in P_{CO_2} are unknown. Second, certain genetic diseases, e.g. cystic fibrosis (CF), are expressed in airway epithelia and may affect regulation of pH_i. The CF gene defect affects several ion transport mechanisms in airway epithelial cell membranes, including apical Na^+ channels (Boucher, Stutts, Knowles, Cantley & Gatzy, 1986; Willumsen & Boucher, 1991) and Cl^- channels (Knowles, Stutts, Spock, Fischer, Gatzy & Boucher, 1983;

Cotton, Stutts, Knowles, Gatzy & Boucher, 1987; Willumsen & Boucher, 1989*a*; Willumsen, Davis & Boucher, 1989*b*) and possibly in membrane pathways for sulphate transport (Cheng, Boucher & Boat, 1987). Based on this evidence, it is possible that plasma membrane paths involved in proton transport and pH_i regulation are likewise affected by CF.

The specific aims of this study were to measure pH_i of normal and CF cells and to explore effects of intracellular acidification and alkalinization on major ion transport functions of the epithelium. Therefore pH-sensitive microelectrodes were used because this technique simultaneously provides information on membrane potentials and voltage divider ratios between apical and basolateral membranes which allow calculation of thermodynamic driving forces for the flow of protons across the two cell membranes.

METHODS

Cell cultures

Primary cultures of normal and CF human nasal epithelial cell cultures were employed in the present study. Tissue cultures and Ussing chamber technique were previously described (Yankaskas, Cotton, Knowles, Gatzy & Boucher, 1985; Willumsen, Davis & Boucher, 1989*a*). In brief, human nasal epithelial cells were isolated and seeded onto collagen matrices which were affixed to the bottom of plastic culture cups. After five to ten mitotic cycles the cells formed a confluent layer. Occurrence of confluence was associated with emergence of a significant transepithelial resistance and potential difference (PD). Cultures were studied within 1 day of development of maximal PD.

Open circuit Ussing chamber technique

The tissue culture cup containing the cell culture was placed in a modified mini-Ussing chamber allowing vertical impalement with microelectrodes. Transepithelial potential (V_t) was measured with a pair of calomel electrodes connected via agar salt bridges to the two half-chambers. Current pulses (ΔI) of 1–10 μA (0·5 s) were passed transepithelially by a pair of Ag^+ electrodes connected to a stimulus isolation unit (World Precision Instruments (WPI) 305, New Haven, CT, USA) and a pulse generator (WPI). From these pulses the fractional apical membrane resistance (fR_a) was determined from the equation:

$$fR_a = \frac{R_a}{R_a + R_b} = \frac{\Delta V_a}{\Delta V_t}, \tag{1}$$

where R_a and R_b are the resistances of the apical and basolateral membranes and ΔV_a and ΔV_t are the deflections in apical membrane potential (V_a) and V_t caused by the transepithelial current pulses. The basolateral membrane potential (V_b) is the difference between V_a and V_t. The equivalent short circuit current was calculated as

$$I_{eq} = V_t/R_t, \tag{2}$$

where R_t is the transepithelial resistance calculated as $\Delta V_t/\Delta I$.

Extracellular pH was measured with a small pH electrode (SA-2, WPI) connected to a pH meter (MP-12, Lazar research instruments, Los Angeles, CA, USA). To vary P_{CO_2} of the luminal solution the preparation was perfused with either gassed (95% O_2, 5% CO_2) or non-gassed KBR solution. When gassed solutions were employed, additional airflow was led directly into the luminal chamber to embed the preparation in a gas envelope equal to that gassing the perfusion solution.

pH-sensitive double-barrelled microelectrodes

Double-barrelled microelectrodes were fabricated as previously described (Willumsen *et al.* 1989*a*). In brief, electrodes were made from fibre-containing glass capillaries (Clark Electromedical Instruments, Reading, UK). Upon silanization (dimethyl-dichloro-silane, Fluka, St Louis, MO, USA) the pH-sensitive barrel was backfilled with a droplet of neutral protonophore (4-nonadecylpyridine, Cocktail A, Cat. No. 95297, Fluka) and subsequently filled with 3 M-KCl

solution adjusted to pH 7·00. The reference barrel was backfilled with 0·5 or 3 M-KCl solution and its resistance was 50–200 MΩ (3 M-KCl) indicating a tip diameter of ~0·1 μm as verified by scanning electron micrographs (Willumsen *et al.* 1989*a*). The sensitivity (*S*) of the pH-sensitive microelectrode was 58·0 ± 1·8 mV (pH unit)$^{-1}$ ($n = 24$) between pH 6 and 8 when measured on backgrounds of 100 mM-NaCl or KCl solutions at 25 °C. For an ideal H^+-selective electrode *S* equals 59 mV (pH unit)$^{-1}$ at 23 °C and 61 mV at 37 °C.

Intracellular pH was calculated from the equation:

$$pH_i = pH_o - (\Delta V_H - V_a)/S, \quad (3)$$

where pH_o is pH of the luminal Ringer solution and ΔV_H the change in signal from the pH sensitive microelectrode upon an impalement.

The Nernstian slope of the pH-sensitive electrodes indicates that Na^+ or K^+ ions did not significantly interfere with the pH-sensitive resin as was expected based on reported magnitudes of selectivity coefficients for this protonophore (2×10^{-9} for Na^+; 2×10^{-10} for K^+; Chao, Ammann, Oesch, Simon & Lang, 1988). The selectivity coefficient for NH_4^+ was $4{\cdot}1 \times 10^{-10}$ when calculated from the Eisenmann–Nicolsky equation (see Willumsen & Boucher, 1991). Generally response times of pH-sensitive microelectrodes were less than 10 s.

The equilibrium potential for H^+ flow across the apical membrane (E_a^H) was calculated from the equation:

$$E_a^H = k(pH_i - pH_o), \quad (4)$$

where $k = F \ln(10)/(RT)$; *F*, *R* and *T* have their usual meanings. The thermodynamic equilibrium pH_i for a given membrane potential (V_a) is given by

$$pH_i^{eq} = (V_a/k) + pH_o. \quad (5)$$

Thermodynamic driving forces for H^+ flow across apical and basolateral membranes ($\Delta\tilde{\mu}_a^H/F$ and $\Delta\tilde{\mu}_b^H/F$) were determined from

$$\Delta\tilde{\mu}_a^H/F = V_a - E_a^H = V_a - k(pH_i - pH_o), \quad (6)$$

$$\Delta\tilde{\mu}_b^H/F = -V_b + E_b^H = -V_b - k(pH_o - pH_i). \quad (7)$$

$\Delta\tilde{\mu}_a^H/F$ is outwardly directed favouring proton excretion when positive. Across the basolateral membrane, proton flow is directed from the serosal side into the cell when $\Delta\tilde{\mu}_b^H/F$ is positive.

Sign conventions and statistics

Throughout the paper, V_a is referenced to the luminal bathing solution, i.e. $V_a = \psi_i - \psi_o$, where ψ_i and ψ_o are the intracellular and luminal (outer) bath electrical potential, respectively. V_b is referenced to the intracellular potential, i.e. $V_b = \psi_s - \psi_i$, where ψ_s is the electrical potential of the serosal bathing solution. V_t is referenced to the serosal bath potential.

Changes in parameters were statistically analysed by standard Student's *t* test. Results are given as means ± S.E.M.

Ringer solution compositions

The composition of the Krebs bicarbonate Ringer solution (KBR) was (mM): 140 Na^+, 120 Cl^-, 5·2 K^+, 25 HCO_3^-, 2·4 HPO_4^{2-}, 0·4 $H_2PO_4^-$, 1·1 Ca^{2+}, 1·2 Mg^{2+} and 5 glucose. Bath pH was maintained at 7·4 by bubbling with a 95 % O_2, 5 % CO_2 gas mixture. HEPES-buffered bicarbonate-free Ringer (HR) was identical to KBR except that HCO_3^- was substituted by Cl^- and 20 mM of HEPES (Sigma). HR was titrated to pH 7·40 with NaOH. Na^+-free HR was identical to HR except that all Na^+ was substituted by *N*-methyl-D-gluconamine (NMDG).

In the ammonium prepulse experiments, 20 mM-NH_4Cl was added to the Ringer solution. This addition did not affect the pH of the solution.

In the amiloride experiments, 10^{-3} M-amiloride (a gift from Merck, Sharp & Dohme, West Point, PA, USA) was added to the Ringer solution.

RESULTS

Intracellular pH of normal and CF preparations

To compare basal intracellular pH, membrane potentials and fR_a of normal and CF airway cell cultures, we employed double-barrelled pH-sensitive microelectrodes. Sequences of illustrative impalements from normal and CF preparations exposed to

bilateral KBR are depicted in Fig. 1. In both normal and CF, pH_i varied little between cells of a single preparation as well as between different preparations despite large variations in membrane potentials. Values for transepithelial, intracellular bioelectric parameters, and pH_i are summarized in Table 1. As previously reported

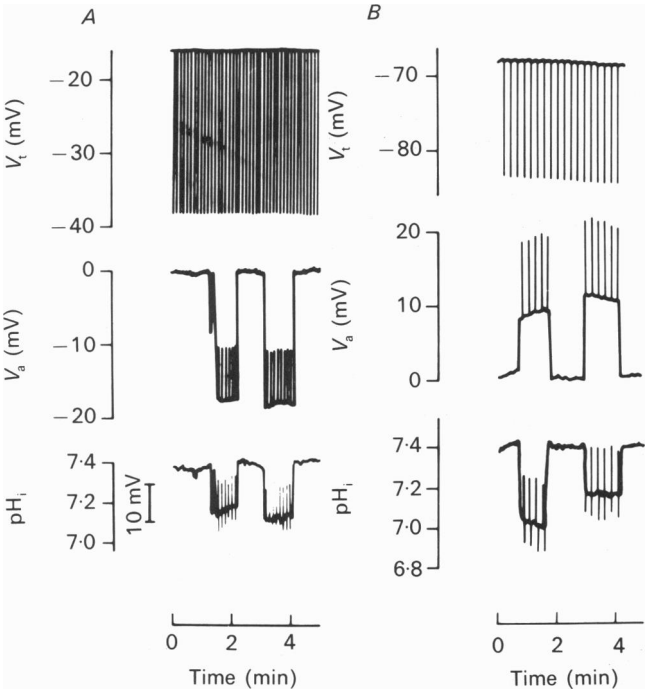

Fig. 1. Examples of impalements of human nasal epithelial cells with double-barrelled pH-sensitive microelectrodes. *A*, impalements of a normal preparation. *B*, impalements of a CF preparation. Note the difference in the magnitude of V_t (upper trace) and in the polarity of V_a (middle trace).

(Willumsen *et al.* 1989*a*, *b*), CF preparations were characterized by a significantly more depolarized apical membrane and larger V_t and I_{eq} than normal preparations. The anomalous values of V_a, V_t and I_{eq} in CF preparations are consequences of an increased absolute Na^+ permeability and a reduced absolute Cl^- permeability of the apical membranes (Willumsen & Boucher, 1989*b*).

Despite striking differences in bioelectric parameters between normal and CF preparations, pH_i was not abnormal in CF cells.

Effect of removing luminal CO_2 on solution pH, bioelectric properties and intracellular pH

When gassing of the luminal bathing Ringer solution (KBR) with 5% CO_2 was stopped, CO_2 quickly escaped from the Ringer solution. The subsequent alkalinization of the luminal medium to approximately pH 8 was accompanied by marked changes

TABLE 1. Summary of intracellular pH, membrane potentials and electrochemical driving forces for protons in normal ($n = 15$) and CF ($n = 13$) human nasal epithelial cell preparations

	V_t	V_a (mV)	V_b	R_t (Ω cm^2)	fR_a	I_{eq} (μA cm^{-2})	pH_i	ΔF_a^H (mV)	ΔF_b^H
Normal	-17.9 ± 1.6	-18.6 ± 2.0	-36.5 ± 2.0	253 ± 15	0.40 ± 0.04	-72.9 ± 6.7	7.15 ± 0.02	-4.1 ± 2.7	22.0 ± 2.6
CF	-45.9 ± 6.7	2.9 ± 5.4	-43.0 ± 2.7	373 ± 47	0.44 ± 0.04	-130.3 ± 16.5	7.11 ± 0.05	19.9 ± 7.1	26.0 ± 3.4
	**	**	n.s.	*	n.s.	**	n.s.	**	n.s.

Values are means $\pm$ S.E.M.
* $P < 0.025$; ** $P < 0.01$; n.s., no significance.

in transepithelial properties of both normal and CF preparations. Figure 2 depicts the changes in transepithelial parameters during a 5 min removal of luminal CO_2 from a normal human nasal preparation. Whereas V_t changed little, I_{eq} increased from 24 to 32 μA cm^{-2} and R_t was reduced from 305 to 255 Ω cm^2. Reintroduction

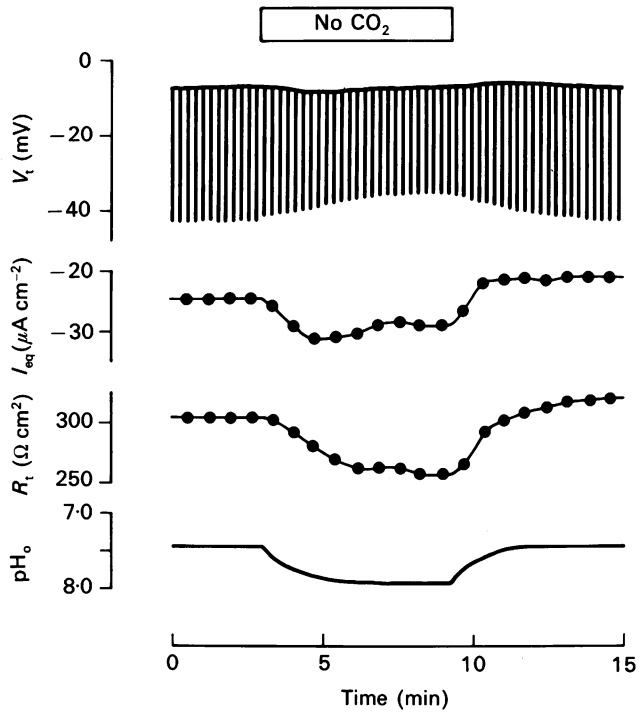

Fig. 2. Effect of elimination of CO_2 in the luminal bathing solution of a normal human nasal epithelial cell culture. Although this manoeuvre had little effect on V_t (upper trace) it elicited significant increases in transepithelial current and reduction in resistance (middle traces) and led to an alkaline shift in pH of the luminal bathing solution (lower trace).

of CO_2 in the luminal bathing Ringer solution led to recovery of pH of the Ringer solution and of all transepithelial bioelectric parameters within a few minutes.

To further investigate the effect of CO_2 removal from the lumen on intracellular bioelectric properties and pH_i, we repeated the CO_2 removal protocol and impaled epithelial cells with pH-sensitive microelectrodes. A sequence of impalements of normal cells during a CO_2 removal experiment is shown in Fig. 3 and the results summarized in Table 2*A*. Characteristically, increase in I_{eq} and reduction of R_t were associated with increase in fR_a and hyperpolarization of V_b.

The effects were qualitatively similar in CF preparations. The response of four CF cultures are collected in Table 2*B*. The effect of CO_2 removal during a continuous impalement of a CF preparation is depicted in Fig. 4. Intracellular pH increased from 7·2 to 7·6 in response to removal of luminal CO_2 while extracellular pH increased from

7·4 to 8·0. Like normal preparations, I_{eq} increased and was associated with an increase in fR_a and a hyperpolarization of V_b.

The effect of luminal CO_2 removal on I_{eq} in all normal and CF preparations is summarized in Fig. 5. It appears that intracellular alkalosis invariably was associated with an increase in I_{eq}.

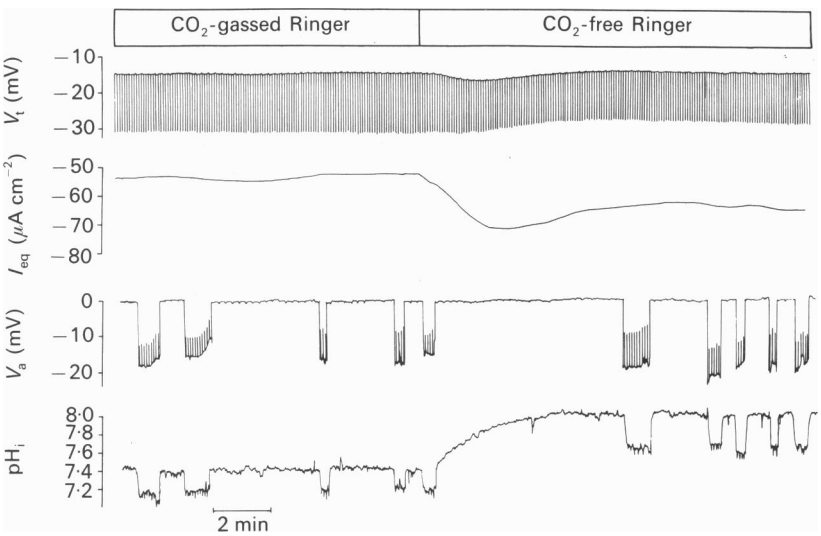

Fig. 3. Sequence of impalements with a pH-sensitive microelectrode of a normal human nasal epithelial culture preparation during a luminal CO_2 elimination protocol. Note that luminal pH (lower trace, baseline) shifts from 7·4 to 7·8 when CO_2 tension of the solution is reduced from 38 mmHg to approximately zero.

Acid loading – effect on intracellular bioelectric properties

To investigate the effects of intracellular acidosis on ion transport properties at the single membrane level, we utilized an NH_4Cl 'prepulse' technique (Roos & Boron, 1981). Preliminary experiments revealed that serosal application of NH_4Cl resulted in a more pronounced intracellular acidification than did luminal application (see below). Furthermore, only a minor initial alkalinization was observed when NH_4Cl was administered to the serosal side, whereas a substantial transient intracellular alkalinization was characteristically observed when NH_4Cl was administered to the luminal surface. A similar sidedness in the effect of ammonium administration was reported from frog skin epithelium by Harvey *et al.* (1988), and it probably indicates that the basolateral membrane has a relatively high permeability for NH_4^+ as compared to the apical membrane.

For the NH_4Cl prepulse experiments designed to acid-load the cells, preparations were exposed to a HEPES-buffered (pH 7·4), HCO_3^--free Ringer solution. Under these conditions, steady-state bioelectric parameters were similar to those observed under control conditions (bilateral KBR). During the NH_4Cl protocol, preparations were exposed to 20 mM-NH_4Cl on the serosal surface for 4 min followed by a return to HR. We monitored pH_i, V_a and V_b, and fR_a with double-barrelled pH microelectrodes.

TABLE 2. Effect of alkalinization of the luminal bathing Krebs bicarbonate Ringer solution by removing CO_2 on intra- and extracellular pH and bioelectric properties of normal ($n = 4$) and CF ($n = 4$) human nasal epithelium

	pH_o	pH_i	V_t	V_a (mV)	V_b	R_t (Ω cm^2)	I_{eq} (μA cm^{-2})	fR_a
				A Normal				
CO_2	7·40	7·16 ± 0·04	−14·4 ± 0·8	−20·3 ± 3·3	−34·6 ± 3·5	250 ± 16	−58 ± 5	0·42 ± 0·07
No CO_2	7·91 ± 0·10	7·42 ± 0·06	−14·0 ± 1·1	−24·3 ± 3·0	−38·3 ± 3·3	207 ± 20	−70 ± 9	0·48 ± 0·06
Diff.	0·51 ± 0·10	0·27 ± 0·03	0·4 ± 0·4	−4·0 ± 1·2	−3·7 ± 1·3	−44 ± 5	−12 ± 5	0·07 ± 0·03
$P <$	0·01	0·005	n.s.	0·025	0·05	0·005	0·05	0·05
				B Cystic fibrosis				
CO_2	7·40	7·12 ± 0·05	−59·4 ± 11·4	11·5 ± 10·8	−47·9 ± 1·9	460 ± 131	−144 ± 33	0·42 ± 0·05
No CO_2	7·93 ± 0·10	7·40 ± 0·03	−56·3 ± 11·2	5·8 ± 11·0	−50·8 ± 1·1	373 ± 103	−163 ± 32	0·52 ± 0·05
Diff.	0·53 ± 0·10	0·28 ± 0·05	3·1 ± 1·0	−5·8 ± 1·7	−2·9 ± 1·0	−87 ± 30	−19 ± 2	0·09 ± 0·02
$P <$	0·01	0·01	0·025	0·025	0·05	0·05	0·005	0·025

Values are means ± S.E.M.
n.s., no significance.

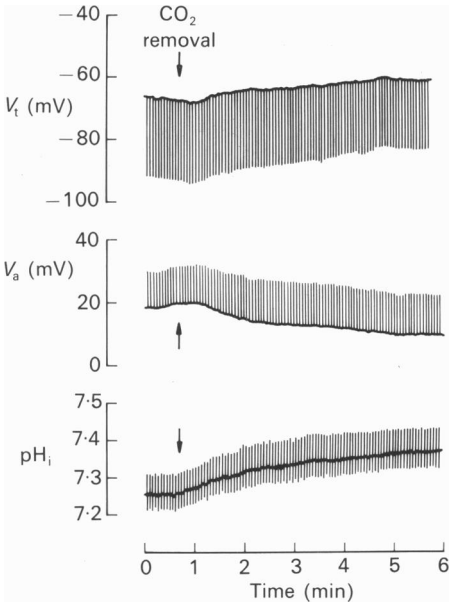

Fig. 4. Continuous tracing with pH-sensitive microelectrode during CO_2 elimination from a CF human nasal epithelial cell culture. Note the very high V_t (−66 mV) and the positive V_a (+20 mV).

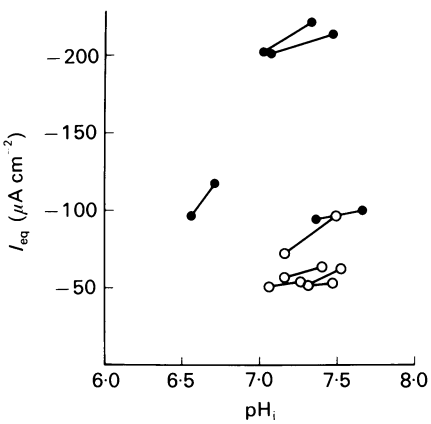

Fig. 5. Summary of the effect of CO_2 elimination in the luminal solution on five normal (○) and four CF (●) human nasal cell preparations. For every pair of points connected by a line segment, the point to the left represents the control state (CO_2-gassed luminal solution) and the point to the right represents the situation with luminal CO_2 removed. In all cases CO_2 removal led to an intracellular alkalinization.

Importantly, in this experimental protocol, extracellular pH was not affected. A typical response to this manoeuvre is shown in Fig. 6. With no exceptions the decrease in pH_i was associated with a reduction in I_{eq} (Fig. 7). As summarized in Table 3, other effects induced by acidification were that V_t decreased, R_t increased,

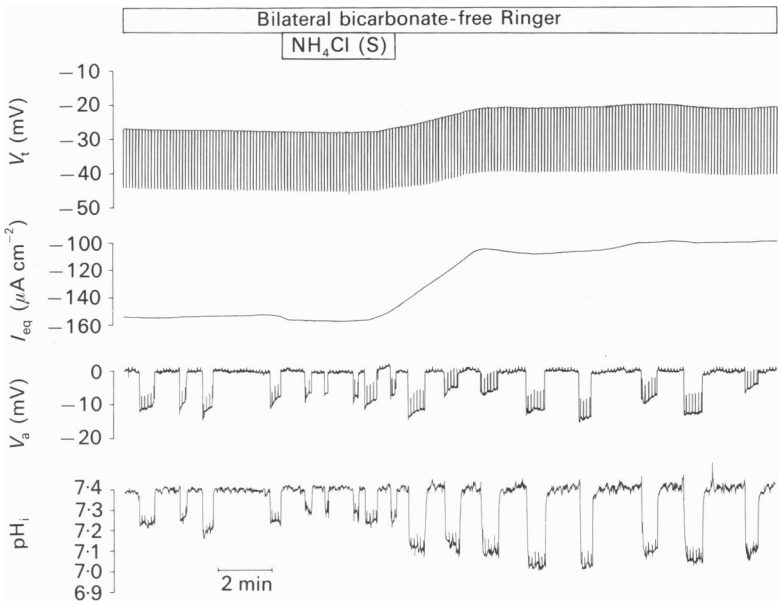

Fig. 6. Intracellular acidification of human nasal epithelial cells by a brief (4 min) exposure to 20 mM-NH_4Cl on the serosal side(s). Large reductions in V_t and I_{eq} were observed in parallel with an intracellular acidic shift from pH 7·25 to 6·95 as measured by a sequence of microelectrode impalements.

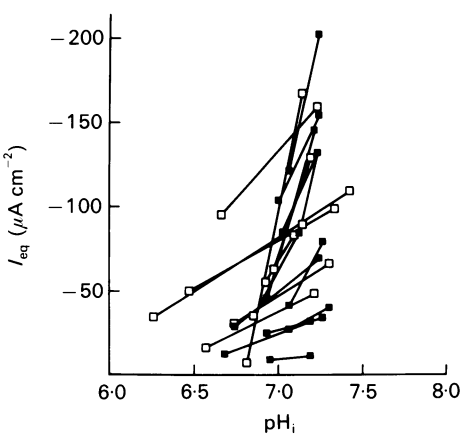

Fig. 7. Summary of the effect of intracellular acidification by ammonium as observed in nine normal (□) and ten CF (■) human nasal epithelial cells. For every pair of points connected by a line segment, the point to the right represents the control value and the point to the left represents the value obtained during intracellular acidification.

fR_a decreased and V_b depolarized, i.e. changes were in general opposite to those observed upon alkalinization (Table 2). No differences were noted in the response between normal and CF preparations (Table 3). However, subsequent recovery of

TABLE 3. Effect of a 4 min 20 mM-NH_4^+ prepulse on the serosal side on intracellular pH and bioelectric properties in normal ($n = 6$) and CF ($n = 7$) human nasal epithelium

	pH_o	pH_i	V_t	V_a (mV)	V_b	R_t (Ω cm^2)	I_{eq} (μA cm^{-2})	fR_a
				A Normal				
Control	7·40	7·18 ± 0·05	−20·3 ± 3·4	−18·5 ± 2·6	−38·8 ± 2·6	218 ± 25	−95 ± 17	0·40 ± 0·03
NH_4^+	7·40	6·80 ± 0·04	−7·6 ± 1·9	−9·0 ± 2·1	−16·6 ± 3·4	244 ± 21	−31 ± 7	0·28 ± 0·02
Diff.	0	−0·39 ± 0·08	12·8 ± 3·4	9·5 ± 2·6	22·3 ± 4·9	26 ± 18	64 ± 20	−0·12 ± 0·01
$P <$	—	0·01	0·01	0·025	0·01	n.s.	0·05	0·005
				B Cystic fibrosis				
Control	7·40	7·21 ± 0·02	−50·4 ± 6·1	6·0 ± 5·6	−44·4 ± 3·5	493 ± 48	−111 ± 19	0·45 ± 0·06
NH_4^+	7·40	6·79 ± 0·08	−32·9 ± 5·1	6·0 ± 3·2	−27·1 ± 4·5	540 ± 54	−67 ± 13	0·34 ± 0·05
Diff.	0	−0·42 ± 0·07	17·5 ± 2·6	0·0 ± 3·1	17·4 ± 2·8	47 ± 14	44 ± 7	−0·11 ± 0·03
$P <$	—	0·005	0·005	n.s.	0·005	0·025	0·005	0·025

Values are means ± S.E.M.
n.s., no significance.

pH_i following intracellular acidification was significantly faster in CF than in normal preparations. Thus, pH_i and I_{eq} recovered within 45 ± 6 min ($n = 6$) in normal and within 14 ± 3 min in CF ($n = 8$) preparations after removal of NH_4Cl.

Effect of intracellular alkalinization by luminal NH_4^+ exposure

Intracellular alkalinization without any change in extracellular pH could be achieved by exposure to NH_4Cl in the luminal bathing solution. Figure 8 depicts a

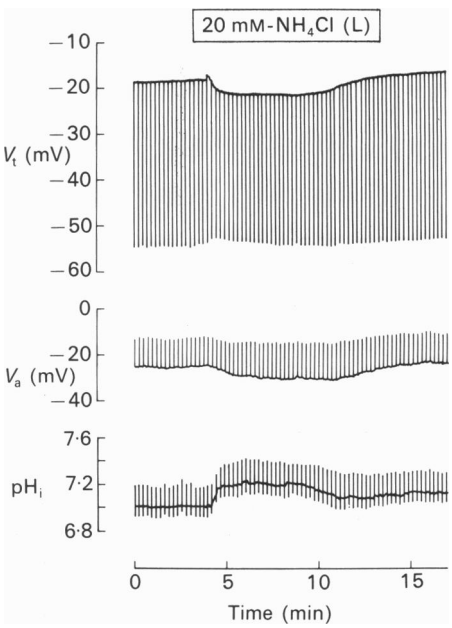

Fig. 8. Intracellular alkalinization of a normal human nasal epithelial cell accomplished by applying 20 mM-NH_4Cl to the luminal (L) bathing solution. Note that as in the CO_2 elimination experiments, V_a shifts towards more negative values, transepithelial resistance (height of voltage deflections on the V_t trace) is reduced and pH_i increases.

continuous impalement of a normal nasal epithelial cell during luminal exposure to 20 mM-NH_4Cl (in HR). Upon NH_4Cl exposure the cell alkalinized by approximately 0·2 pH units, indicating that predominantly NH_3 (rather than NH_4^+) entered the cells across the apical membranes. The increase in pH_i was accompanied by changes in bioelectric parameters which were qualitatively similar to those observed during CO_2 removal, i.e. hyperpolarization of V_t and V_a, decrease in R_t, increase in I_{eq} and increase in fR_a. During this protocol extracellular pH remained unchanged and we could therefore exclude the possibility that luminal alkalinization in CO_2 removal experiments directly caused the changes in bioelectric properties.

Effect of luminal Na^+-free media and amiloride

To test for presence and possible role of an active apical Na^+–H^+ exchanger that may contribute to pH_i regulation in response to changes in pH_i, we studied the effect of exposing the luminal surface of cell cultures to Na^+-free Ringer solutions (HR) on pH_i within 15 min. An illustrative tracing from a CF preparation is shown in Fig. 9.

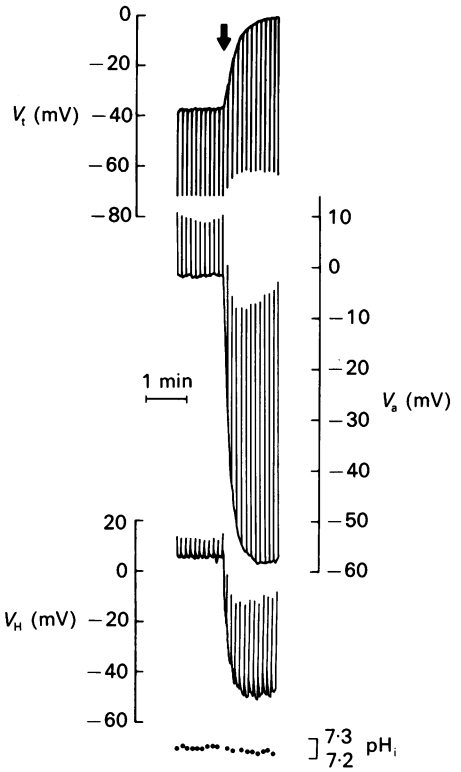

Fig. 9. Effect of removal of Na^+ from the luminal bathing solution (HEPES-buffered Ringer solution) on V_t (upper trace), V_a (upper middle trace), V_H (lower middle trace) and pH_i (lower trace). Luminal Na^+ was removed at the arrow. No effect on pH_i was detected.

TABLE 4. Effect of Na^+-free luminal bathing solution on steady-state transepithelial potential, apical membrane potential and intracellular pH

	V_t (mV)	V_a (mV)	pH_i	n
Normals				
Control	−16·8±4·5	−21·5±2·8	7·16±0·05	4
Na^+ free	−3·8±3·6*	−34·5±6·1*	7·24±0·06	4
CF				
Control	−25·0±6·1	−13·3±6·4	7·16±0·06	4
Na^+ free	−0·5±0·5*	−40·5±8·8*	7·11±0·10	4

* Significantly different from control value ($P < 0·01$).

Effects of luminal Na^+ removal in four normal and four CF preparations are listed in Table 4. While V_t was reduced to 4 mV in normals and virtually abolished in CF preparations and the apical membrane hyperpolarized by 13 and 27 mV in normal and CF preparations, respectively, no significant shift in pH_i was detected in either preparation type.

In another set of experiments, we exposed four normal and four CF cultures to 10^{-3} M-luminal amiloride in HR. Figure 10 depicts a typical experiment. The effect

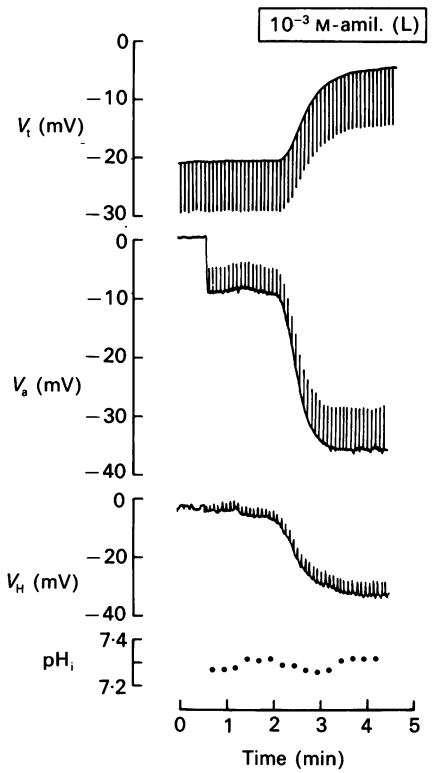

Fig. 10. Effect of amiloride (10^{-3} M) in the lumina HEPES-buffered Ringer solution on V_t (upper trace), V_a (upper middle trace), V_H (lower middle trace) and pH_i (lower trace). Note that pH_i is not affected by amiloride.

TABLE 5. Effect of amiloride (10^{-3} M) on steady-state transepithelial potential, apical membrane potential and intracellular pH

	V_t (mV)	V_a (mV)	pH_i	n
Normals				
Control	−15·3±2·5	−14·5±2·7	7·34±0·04	4
Amiloride	−4·1±0·7*	−31·0±5·1*	7·32±0·07	4
CF				
Control	−42·5±11·6	2·05±5·1	7·11±0·08	4
Amiloride	−0·6±0·7*	−32·8±6·3*	7·04±0·10	4

* Significantly different from control value ($P < 0·01$).

on V_t and V_a was similar to that of removing Na^+ from the lumen: large reductions in V_t and hyperpolarization of the apical membrane. However, no shift in pH_i occurred (Table 5).

To test if an apical Na^+–H^+ exchanger was active when the epithelial cells were acidified to a pH_i value considerably lower than the set-point for the Na^+–H^+ exchanger as reported from, for example, lymphocytes (Grinstein, Goetz, Cohen, Rothstein & Gelfand, 1985), we preacidified the cells by a serosal NH_4^+ prepulse to

approximately 6·9 and subsequently exposed the luminal surface of the preparation to 10^{-3} M-amiloride (not shown). In two normal and two CF preparations, we observed no effect on subsequent pH_i recovery when preparations were exposed to 10^{-3} M-amiloride at the time of peak intracellular acidification. Thus, normal and CF preparations alkalinized by $0{\cdot}39 \pm 0{\cdot}18$ and by $0{\cdot}35 \pm 0{\cdot}012$ pH units within 30 and 15 min, respectively.

DISCUSSION

The notion of abnormal intracellular pH in CF epithelial cells is raised by studies that conclude that the CF gene-product, the cystic fibrosis transmembrane regulator (CFTR), probably represents a certain type of native epithelial Cl^- channel (Kartner, Hanrahan, Jensen, Naismith, Sun, Ackerly, Reyes, Tsui, Rommens, Bear & Riordan, 1991; Anderson, Rich, Gregory, Smith & Welsh, 1991). This relationship may be a direct consequence of channel-like activity. For example, pH_i may be affected by lack of a functional Cl^- channel if this channel also serves as a transport pathway for bicarbonate. CFTR exhibits homology with mammalian multidrug resistance (MDR) P-glycoprotein (Riordan, Rommens, Kerem, Alon, Rozmahel, Grzelczak, Zielinski, Lok, Plavsic, Chou, Drumm, Ianuzzi, Collins & Tsui, 1989), and recent reports have linked pH_i to the degree of multidrug resistance in human cancer cell lines (Lyon, Cohen, Faustino, Megnin & Meyers, 1988; Keizer & Joenje, 1989; Boscoboinik, Gupta & Epand, 1990). Whether this effect represents channel-like activity of MDR P-glycoprotein, or other activities, is unknown.

Basal intracellular pH

The present study demonstrated that despite a large difference in V_a between normal ($-18{\cdot}6 \pm 2{\cdot}0$ mV, $n = 15$) and CF ($2{\cdot}9 \pm 5{\cdot}4$ mV, $n = 13$) preparations under control conditions pH_i values were not different in the two preparation types; $7{\cdot}15 \pm 0{\cdot}02$ in normal and $7{\cdot}11 \pm 0{\cdot}05$ in CF preparations (Table 1). These values are characteristic of pH_i in a variety of mammalian epithelial cells (reviewed by Roos & Boron, 1981). Also, they agree well with results reported from human nasal epithelium based on a fluorescent proton probe (Paradiso, Yankaskas & Boucher, 1989).

Due to the difference in membrane potential, protons appeared to be accumulated at or slightly below their thermodynamic equilibrium across apical membranes of normal preparations, whereas H^+ ions were above equilibrium across apical membranes of CF preparations. Considering the measured V_a in normal and CF preparations (Table 1), the theoretical thermodynamic equilibrium pH_i would be 7·08 and 7·45, respectively. Mean driving forces for proton flow across the apical membrane were 4 ± 3 mV directed from lumen to cell in normal and 20 ± 7 mV directed from cell to lumen in CF preparations. Thus, if protons are able to leak across the apical membrane, the net fluxes would be oppositely directed in normal and CF epithelial cells. Significant secretory fluxes of H^+ in CF epithelia due to the relatively large outwardly directed transapical driving force would be expected to lead to excessive acidification of the airway surface liquid. However, no such difference in pH of the fluid lining of airways has been observed *in vivo* (M. R. Knowles, personal communication).

pH_i effects on transepithelial and intracellular parameters

In both serosal and luminal Ringer solutions CO_2-gassed bicarbonate Ringer solution was substituted with a HCO_3^--free HEPES-buffered Ringer solution. This substitution had no effects on steady-state pH_i or bioelectric properties. Therefore, we resorted to other manoeuvres to vary pH_i.

Removal of CO_2 from the luminal bathing KBR solution increased pH of that solution from 7·4 to approximately 8·0; pH_i also shifted in the alkaline direction but less than the extracellular pH, from 7·1 to 7·4, increasing the transapical pH gradient from 0·24 to 0·49 pH units in normal and from 0·28 to 0·53 pH units in CF preparations. The shifts in intra- and extracellular pH were associated with pronounced alterations in the bioelectric properties of both normal and CF preparations (Table 2A and B). Similar effects on electrophysiologic properties were elicited by luminal exposure to NH_4^+ (Fig. 8) in which case extracellular pH was not affected. The increase in fR_a, reduction of R_t, and hyperpolarization of V_b all point towards an increase in basolateral K^+ conductance as the primary electrophysiological response to the induced alkalinization. The increase in I_{eq} is likely to be dominated by change in the basolateral K^+ conductance although a smaller effect on apical Na^+ conductance also is likely (see below).

The effect of the serosal ammonium prepulse protocol on the epithelial cells exposed to HR was a decrease of pH_i from 7·2 to 6·8 (Table 3), and it was associated with marked changes in bioelectric parameters which, taken together, most easily is interpreted as reflecting a reduction in basolateral K^+ conductance. Especially impressive is the reduction of transepithelial I_{eq} by 67% in normal preparations (Table 3A) and by 39% in CF preparations (Table 3B).

The effects of NH_4Cl pulses and removal are likely to reflect direct effects of intracellular pH. Prolonged intracellular acidosis was accomplished by exposure to 20 mM-NH_4Cl in the serosal bathing Ringer solution and this addition constituted a 13% increase in total osmolality of the Ringer solution. However, previously we have shown that serosal hyperosmolality accomplished by addition of 150 mM-sucrose or NaCl has no significant effect on bioelectric parameters of either normal or CF preparations (Boucher & Willumsen, 1988, 1989) demonstrating that the NH_4Cl-induced responses were not due simply to changes in osmolality. Also, serosal NH_4^+ eliminated the possible effect of interference between NH_4^+ and the pH-sensitive resin. However, as ammonium ions are expected to reach a high intracellular concentration at equilibrium, interference is a potentially more serious problem intracellularly. Fortunately, the selectivity of the resin for ammonium is sufficiently low (see Methods) to avoid any significant error. Finally, despite the possibly large intracellular ammonium concentrations during the acidification experiments, all induced changes in bioelectric parameters were fully reversible within 5–60 min, indicating no serious permanent cell damage.

Effects of pH_i on basolateral membrane ion transport systems

Our data show that in both normal and CF human airway epithelia intracellular alkalinization is associated with activation of a basolateral conductance whereas intracellular acidification leads to inactivation of a basolateral conductance. The

direction of the changes in V_b indicates that the affected conductance is specific for K^+. Figure 11 summarizes changes in V_b and fR_a. Despite large variations in the amplitude of the response to a given pH_i perturbation, it is evident that the basolateral membrane invariably depolarized in response to intracellular acidosis

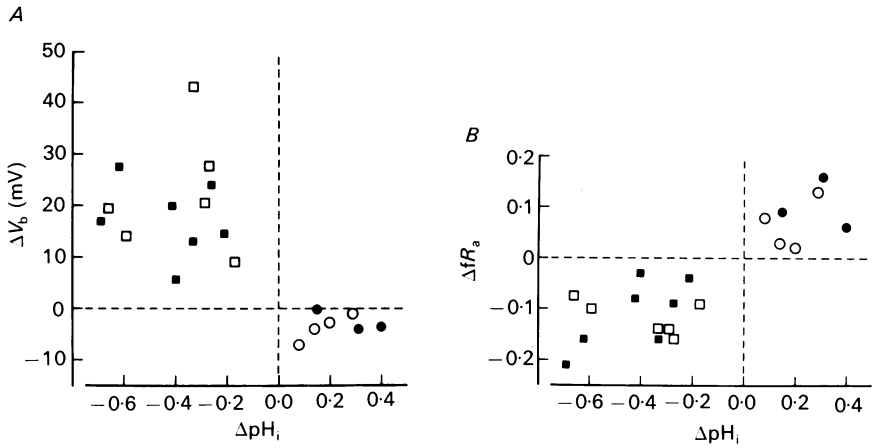

Fig. 11. Changes in V_b (*A*) and fR_a (*B*) as function of the corresponding change in pH_i. Circles refer to CO_2 removal experiments and squares refer to NH_4^+ prepulse experiments. Open symbols represent normal preparations and closed symbols represent CF preparations.

and in general hyperpolarized in response to intracellular alkalosis. Likewise, fR_a invariably decreased in response to acidification and increased in response to alkalinization. Figure 12 summarizes relative changes in I_{eq} observed in alkalinization and acidification experiments.

The finding of covariance between intracellular pH and the activity of cellular ion transport systems is in accordance with data from other preparations. Thus, several studies have suggested that pH_i may regulate the activity of various epithelial and non-epithelial membrane ion transport systems.

Basolateral membranes of human nasal epithelial cells are highly conductive for K^+ and also have a moderate Cl^- conductance (Willumsen *et al.* 1989*a*, *b*). Regulation of basolateral membrane K^+ conductance by pH_i has been reported from frog skin (Harvey *et al.* 1988), bovine retinal pigment epithelium (Keller *et al.* 1986), outer renal medulla (Klærke *et al.* 1987) and rabbit distal colon (Duffey & Devor, 1990). Regulation of K^+ channels by pH_i has been reported from non-epithelial cell membranes as well, for example, pancreatic B-cells (Cook, Ikeuchi & Fujimoto, 1984) and human red cells (Stampe & Vestergaard-Bogind, 1985).

Recently, Ca^{2+}-sensitive K^+ channels in basolateral membrane of normal and CF human airway epithelia were identified with the patch-clamp technique (Kunzelmann, Pavenstädt, Beck, Ünal, Emmrich, Arndt & Greger, 1983*a*; Kunzelmann, Pavenstädt & Greger, 1989*b*), and these channels were not sensitive to pH on the cytosolic side of the patch. One possible explanation for this apparent discrepancy is that these channels are not the ones responsible for the changes in basolateral K^+

conductance induced by shifts in pH_i as it is well known that more than one class of basolateral K^+ channels has been identified in epithelial cells (see review by Lang, Oberleithner, Kolb, Paulmichl, Volkl & Wang, 1988). Another explanation could be that the K^+ channels loose their pH sensitivity upon excision. Patch-clamp studies

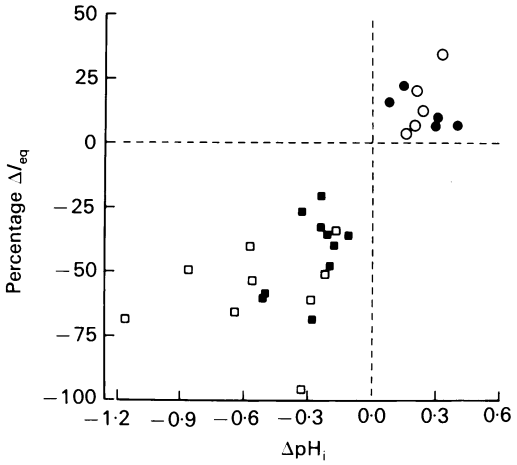

Fig. 12. Summary of the effect on I_{eq} of perturbations of pH_i in either the alkaline (circles) or acidic (squares) direction. Note that changes in I_{eq} are given as a percentage of the control value. Open symbols represent normal and closed symbols represent CF human nasal epithelial cell culture preparations.

of the effect of changes in intracellular pH on different classes of basolateral K^+ channels in airway epithelia are therefore warranted.

Possible effect of pH_i on apical Na^+ channels

The large effects of pH_i on I_{eq} suggest a possible effect on transcellular Na^+ transport mechanisms, i.e. apical Na^+ channels and/or basolateral Na^+–K^+-ATPase. In apical cell membranes of frog skin epithelium, the Na^+ permeability is influenced by pH_i (Harvey *et al.* 1988). Thus, intracellular acidosis led to reduction in apical membrane Na^+ conductance (g_a^{Na}) whereas intracellular alkalosis increased g_a^{Na}. In all cases g_a^{Na} was a sigmoidal increasing function of pH_i with the steepest slope around the physiological pH_i. It was concluded that pH_i is the signal that co-ordinates the ion conductances of the apical and basolateral membranes (the phenomenon of 'cross-talk').

It is not clear whether apical membrane Na^+ permeability and/or the Na^+–K^+ pump were affected by the changes in pH_i in normal human airway epithelial cells because presence of a large g_a^{Cl} precludes simple resolution and analysis of g_a^{Na}. If so, the effect on basolateral K^+ channels apparently dominated the overall response. In the case of CF, in which we have no evidence for conductive paths for ions other than Na^+ in the apical membrane, we can, however, estimate the apical Na^+ permeability (P_a^{Na}) by a method described in a previous paper (Willumsen & Boucher, 1989*b*). Employing data from Table 3B, we can calculate the acidification of cells by NH_4Cl

exposure reduced P_a^{Na} from $9{\cdot}5 \times 10^{-6}$ cm s^{-1} to $7{\cdot}0 \times 10^{-6}$ cm s^{-1}. However, when pH_i was increased by removal of luminal CO_2 (data from Table 2B), the calculated P_a^{Na} remained unchanged $1{\cdot}3 \times 10^{-5}$ cm s^{-1}.

Apparent lack of passive proton paths in the apical membrane

Generally, recovery of pH_i subsequent to intracellular acidification involves the action of a Na^+–H^+ exchange mechanism. Apical Na^+–H^+ exchange has been

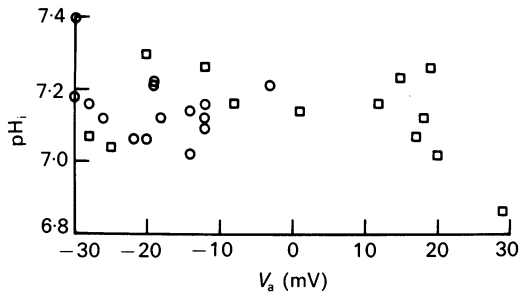

Fig. 13. Lack of correlation between pH_i and apical membrane potential. Circles represent normal preparations and squares represent CF preparations.

demonstrated in more distal airway epithelia (alveolar type II cells; Sano, Cott, Voelker & Mason, 1988; Shaw, Steele, Butcher, Ward & Oliver, 1990). However, in the present study, removal of luminal Na^+ or exposure to amiloride in doses known to inhibit the Na^+–H^+ exchanger in other preparations, did not affect pH_i (Figs 9 and 10, Tables 4 and 5). This could be a consequence of pH_i already being above the set-point for the Na^+–H^+ exchanger. However, exposure to luminal amiloride after intracellular acidification to $pH_i = 6{\cdot}9$ with NH_4^+ prepulse technique (not shown) did not affect the pH_i recovery either.

The simplest explanation for lack of any effect of amiloride or luminal Na^+ removal on pH_i is that the regulatory system responsible for recovery of pH_i, probably a Na^+–H^+ exchanger, is located in the basolateral barrier. This observation is supported by recent data obtained from human nasal epithelial cells by fluorescent proton probe technique (A.M.Paradiso, unpublished results).

There was no correlation between pH_i and V_a (Fig. 13) or V_b (not shown). This observation indicates that pH_i is not markedly influenced by passive electrodiffusive membrane proton transport pathways on either the apical or basolateral barrier.

Comparison of recovery from acid load

Recovery from acid loads generally occurred considerably faster in CF than in normal preparations. Although the experiments of the present study did not reveal the cause of the difference in time course of pH_i recovery process, it must reflect activities independent of H^+ conductances and apical Na^+–H^+ exchange. Similarly, absence of effects of removal of HCO_3^- (shift from KBR to HR) indicates Cl^-–HCO_3^- exchange is not likely to be present. These data may point future investigations to a basolateral Na^+–H^+ exchange activity and its regulation.

Relation to in vivo conditions

The shift in P_{CO_2} of the luminal bathing Ringer solution employed in the acidosis experiments (from 5% to 0·02% corresponding to the CO_2 content of atmospheric air) mimics actual variation in CO_2 content of luminal air mass during a complete breathing cycle. This variation in luminal P_{CO_2} most probably significantly affects pH of the fluid film lining the airway surface (which is only $\sim 15\ \mu m$ thick; Killburn, 1968), regardless of the buffering capacity. The pH of this thin liquid film is likely to be sensitive to any net absorption or secretion of H^+. In accordance with this notion, the present study indicated that H^+ is not exchanged for Na^+ across the apical membrane by an amiloride inhibitable Na^+–H^+ exchanger. Thus, changes in pH of airway surface liquid are probably dominated by changes in ambient P_{CO_2}.

The results also predict that variations in pH_i of airway epithelia may occur *in vivo* in response to shifting luminal P_{CO_2} and that these changes would affect bioelectric properties of the epithelia. The time course of shifts in pH_i and bioelectric parameters was long in these experiments as compared to the duration of a normal breathing cycle ($\sim$ 5–6 s); however, the time constants of the shifts reported here merely reflect time constants of solution changes in the luminal half-chamber. Therefore, it is likely that basolateral K^+ channels *in vivo* undergoes rhythmic variations in conductivity governed by the actual breathing rate. In fact, rhythmic changes in transepithelial potential difference in phase with the breathing cycle have been observed (Knowles, Stutts, Yankaskas, Gatzy & Boucher, 1986).

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